Interferon-inducible genes are major targets of human papillomavirus type 31: Insights from microarray analysis

Yijan E. Chang and Laimonis A. Laimins*
Department of Microbiology-Immunology, Northwestern University Medical School, Chicago, IL 60611, USA

Human papillomavirus (HPVs) are small DNA viruses that infect epithelial tissue. More than 70 subtypes of HPV have been identified to date and they exhibit specific tropism to various region of the body [11, 19,33]. Genital HPVs can be divided to high-risk and low-risk groups [19]. The high-risk genital HPVs (HPV16, 18, 31, 33, and 54) are the etiological agents of cervical cancer, whereas low-risk HPVs (HPV6 and 11) are associated with common genital warts. Cervical cancer is the second most common cancer in women world wide, resulting in 500,000 new cases and 200,000 death every year. Because of the lack of the access to routine Pap smears, cervical cancer remains a devastating disease in developing countries [15].

The genome of HPVs encodes only 8 to 10 proteins. The virus must therefore depend on basic cellular machinery to modulate cellular activities in favor of viral replication. As a result, the interaction of viral proteins with the host proteins is essential for viral replication. The best known examples include the interaction of oncoproteins E6 and E7 of high-risk HPVs with p53 and pRB, respectively. These interactions result in degradation and inactivation of p53 by E6 [12,26, 27], and inactivation of pRB by E7, resulting in altered regulation of E2F-inducible genes [5,8,20]. In addition to E6 and E7, HPV E2 protein has been shown to have transactivation/repression activity by binding to specific recognition sequences [3,21], and E5 protein has been shown to enhance signal transduction through the EGF and PDGF receptors [16]. Such alterations are likely to have significant effects on cellular gene expression. An understanding of the virally induced-changes in cellular gene expression would shed light on HPV viral life cycle and pathogenesis.

The HPV viral life cycle can be divided into at least two stages. The virus establishes latent infection in the basal layer of epidermis following primary infection. Upon keratinocye stratification and differentiation, the viral genome is amplified and late structural genes are synthesized, which leads to virion maturation [7]. Because of the requirement for differentiation at the late stage of viral life cycle, the lack of a good in vitro tissue culture system to propagate the virus has been a major hurdle in HPV research. Recently, a tissue culture model that mimics the latent stage of HPV infection has been developed [7,23]. Cloned HPV 31 and HPV 18 genome have been used to transfect normal human keratinocytes (NHK) resulting in cell lines that exhibit characteristics of basal keratinocytes infected with HPV. Once induced to differentiate, these cell lines are able to activate late viral functions and produce virions [10,23]. This system provides an excellent model to investigate virus-cell interactions under physiologically relevant conditions. We have been interested in examining cellular changes at the transcriptional level during the first stage of infection by HPV31. Our experimental approach has been to compare the transcriptional profile of keratinocytes containing transfected HPV31 genome, with that of normal keratinocytes by microarray analysis [13,32]. In order to avoid any variation resulted from specific genetic background, HPV31 cells were compared with uninfected keratinovytes from the same donor.

Microarray analysis was performed with an array containing 7,075 expressed sequence tags (ESTs), including 4,000 known genes (InCyte Human UniGem V). We observed that expression of most of the ESTs

*Address for correspondence: Department of Microbiology-Immunology, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL 60611, USA. Tel.: +1 312 503 0648; Fax: +1 312 503 0649; E-mail: l-laimins@northwestern.edu.

Disease Markers 17 (2001) 139–142
ISSN 0278-0240 / $8.00 © 2001, IOS Press. All rights reserved

Table 1
Genes whose expression was altered by more than 2.4-fold

Genebank	Gene name	Diff. expr.	Genebank ID	Gene name	Diff. expr.
M33882	myxovirus (influenza) resistance 1	−11.5	X04741	ubiquitin carboxyl-terminal esterase L1	+3.2
X67325	interferon-alpha inducible 11.5 KD protein	−10.1	V00571	corticotropin releasing hormone	+3.1
X03557	interferon-inducible protein 56	−9.6	T91294	ESTs	+3.1
W76205	ESTs	−6.9	X16832	cathepsin H	+2.9
S73288	small proline-rich protein SPRK	−5.4	M69199	human GOS2 gene	+2.9
M20030	human small proline rich protein (sprII)	−5.1	M73548	adenomatosis polyposis coli	+2.9
W73855	ESTs	−3.9	AA085711	ESTs	+2.8
U65590	interleukin 1 receptor antagonist	−3.9	AA477828	ESTs	+2.8
L33404	protease, serine, 6	−3.8	AA705034	ESTs, weakly similar to reverse transcriptase	+2.7
M34715	pregnancy specific beta-1-glycoprotein 7	−3.6	R55750	ESTs	+2.7
X04470	secretory leukocyte protease inhibitor	−3.6	AA018443	ESTs	+2.7
M87284	2'-5' oligoadenylate synthetase 2	−3.5	W67951	ESTs	+2.7
AA741307	ESTs	−3.5	AA731863	ESTs	+2.7
M97935	signal transducer and activator of transcription-1		AA134111	ESTs	+2.6
	α/β	−3.5	AA705184	ESTs	+2.6
Y00630	plasminogen activator inhibitor type II	−3.4	AA524538	succinate CoA ligase, ADP-forming, beta subunit	+2.6
L33930	human CD24 gene	−3.3	AA633231	ESTs	+2.6
J04164	interferon-inducible protein 1-8U	−3.3	AA040834	ESTs, weakly similar to collagens	+2.6
U50931	defensin, beta-1	−3.2	H18233	ESTs	+2.5
X59770	interleukin 1 receptor, type II	−3.2	AA150502	ESTs	+2.5
M95787	transgelin	−3.2	AA575973	ESTs	+2.5
X99133	neutrophil gelatinase-associated lipocalin precursor	−3.1	AI033548	ESTs	+2.5
X74330	primase, polypeptide 1	−3.0	W15253	ESTs	+2.5
X56807	desmocollin 2	−2.9	N57571	ESTs	+2.5
X82200	human Staf50 mRNA	−2.8	R55697	ESTs	+2.5
M30818	myxovirus (influenza) resistance 2	−2.8	D83884	ESTs	+2.5
U92314	sulfotransferase family 2B, member 1	−2.7	N22132	ESTs	+2.5
U09364	zinc finger protein 136	−2.6	AA425325	ESTs	+2.5
X04327	2,3-bisphosphoglycerate mutase	−2.6	AA805921	ESTs	+2.5
U72882	interferon-inducible leucine zipper protein (IFP35)	−2.6	AA527448	ESTs	+2.5
X86809	phosphoprotein enriched in astrocytes 15	−2.6	AA134926	ESTs	+2.4
Z85996	CKI (p21)	−2.6	AA683531	ESTs	+2.4
L06895	MAX dimerization protein (Mad)	−2.5	AA648117	ESTs	+2.4
M22612	trypsin1	−2.5	AA733074	ESTs, weakly similar to C15H9.5 (C.elegans)	+2.4
U79725	human A33 antigen precursor	−2.5	AA280262	ESTs	+2.4
AJ000480	C8FW phosphoprotein	−2.5	N52534	ESTs	+2.4
M77830	desmoplakin I and II	−2.5	U39905	solute carrier family 18	+2.4
X89960	mitochondrial capsule selenoprotein	−2.5	AA056410	ESTs, weakly similar to IgE receptor beta subunit	+2.4
X57348	stratifin	−2.5	AA190841	ESTs	+2.4
M23263	androgen receptor	−2.4	AA846757	ESTs	+2.4
AA100757	ESTs	−2.4	H98977	ESTs	+2.4
AF013970	myeloid translocation gene-related protein 1	−2.4	N49233	ESTs	+2.4
AB001928	cathepsin L2	−2.4	AA557324	ESTs, highly similar to cytochrome P450 IVA2	+2.4
X13916	low density lipoprotein-related protein 1	−2.4	N51427	ESTs	+2.4
L34155	laminin, alpha 3	−2.4	AI025984	ESTs	+2.4
V01512	v-fos FBJ murine osteosarcoma viral oncogene		R68857	ESTs	+2.4
	homolog	−2.4	J04456	galectin 1	+2.4
X97198	receptor phosphatase PCP-2	−2.4	N34956	frizzled homolog 7	+2.4
			U04343	T lymphocyte activation antigen CD86 precursor	+2.4

Diff. Expr., Differential Expression. "−" indicates suppression and "+" indicates activation of expression in HPV31 cells.

was not changed by the presence of HPV genes. However, the expression of 178 ESTs (2.5% of total ESTs examined) was increased by between 2 to 3 fold by HPV31, with no activation more than 3.2 fold observed. In addition, the expression of 150 ESTs (2.1%) was decreased by at least two fold in HPV31 cells, with the largest change being 11.5 fold. The annotations of genes that were affected by more than 2.4 fold are listed in Table 1. The entire list of genes examined and the level of differential expression in normal and in HPV31 cells can be found at http://bugs.mimnet.nwu.edu/laimins_lab/chang [4].

From this analysis, the genes that were up-regulated in HPV31 cells were found to be ESTs with unknown functions, and the small number of known genes that were increased at significant level are also listed in Table 1. However, the number of genes in that category is not large enough for us to discern any pattern of activation of specific families of genes. Notably, no E2F, or E2F inducible genes were activated significantly in HPV31 cells.

In contrast to genes that were up-regulated, it was simple to identify families of genes down-regulated in HPV31 cells. These can be divided into three categories: genes that are negative regulators of cell growth, genes that are specifically expressed in keratinocytes, and genes that are induced by interferon (IFN). The first category includes p21, Mad and transgelin. The expression of p21 is dependent on p53, and its reduced expression is consistent with the fact that high-risk HPV E6 protein inactivates p53 and thereby reduces the level of p21 expression [9]. Mad interacts with Max and antagonizes the proliferating effect of Myc/Max complex [1] which may play a role in activating telomerase [30]. Transgelin is a gene that is highly expressed in senescent cells, though the function is still not clear [28]. The second category we found to be repressed includes small proline rich protein SPRK, and SprII, defensin, desmocollin 2, desmoplakin, and stratifin [14,17,22,24, 29,31]. The role of these keratinocyte specific proteins in HPV pathogenesis is not clear. One can envision that by altering the expression of these genes, the cellular environment of the natural host of HPV is therefore changed in terms of response to stress, cell-cell contact, and altered differentiation program.

The third category of genes repressed by HPV includes those involved in the interferon pathway. These genes represent at least 14% of the total genes that were negatively regulated by 2.3 fold or more in HPV31 infected cells. This group includes genes that showed the strongest changes: myxovirus resistance 1 (MxA, 11.5 fold), IFN-α-inducible 11.5 KDa protein (10.1 fold), and IFI 56 (9.6 fold). Among the IFN-inducible genes affected by HPV infection is the major regulator of IFN responsive pathway, Stat-1 (Signal Transducer and Activator of Transcription 1, which was repressed by 3.5 fold) [6]. Down-regulation of the basal level expression of IFN responsive genes in HPV31 cells could be largely the result of reduced basal level of Stat-1 expression. Further analysis has demonstrated that HPV31 cells showed a delayed response to IFN-α in terms of the induction of Stat-1 and MxA RNA. However, upon longer exposure to higher does of IFN-α, the response reached the same level as in uninfected cells. It has been shown that high-risk HPVs interfere with the IFN response in several ways, by blocking interferon synthesis [25], or by blocking the function of Stat-1 post-translationally [2,18]. However, suppression of basal level of Stat-1 expression by HPV has not been reported previously, although Stat-1 activity can be regulated at a post-traslational level. The reduced basal level of Stat-1RNA and protein did provide an advantage for HPV replication by delaying the cellular response to interferon. This delay could be very significant for the virus in evading immune surveillance. It has been postulated that reduced level of Stat-1 may also contribute to tumor progression. We are currently investigating the mechanism of Stat-1 gene suppression. Preliminary data suggested that both Stat-1 promoter inactivation and Stat-1 protein turn over rate may play important roles in regulating Stat-1 activity in HPV31 cells. The role of individual viral genes in these processes is being examined.

These studies demonstrate the utility of applying microarray analysis to HPV positive cells. Such studies can identify new markers of viral infection and potentially prognostic markers of disease progression. A more thorough analysis of biopsy materials and changes induced by other HPV types is needed to make the studies complete.

References

[1] D.E. Ayer, L. Kretzner and R.N. Eisenman, Mad: a heterodimeric partner for Max that antagonizes Myc transcriptional activity, *Cell* **72** (1993), 211–222.

[2] P. Barnard and N.A.J. McMillan, The human papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-α, *Virology* **259** (1999), 305–313.

[3] V. Bouvard, A. Storey, D. Pim and L. Banks, Characterization of the human papillomavirus E2 protein: evidence of trans-activation and trans-repression in cervical keratinocytes, *EMBO Journal* **13** (1994), 5451–5459.

[4] Y.E. Chang and L.A. Laimins, Mcroarray anlaysis identifies interferon-inducible genes and Stat-1 as major transcriptional targets of human papillomavirus type 31, *Journal of Virology* **74** (2000), 4147–4182.

[5] S. Cheng, G.D. Schmidt, T. Murant, T.R. Broker and L.T. Chow, Differentiation-dependent up-regulation of the human papillomavirus E7 gene reactivates cellular DNA replication in suprabasal differentiated keratinocytes, *Genes & Development* **9** (1995), 2335–2349.

[6] J.E.J. Darnell, I.M. Kerr and G.R. Stark, Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins, *Science* **264** (1994), 1415–1421.

[7] S.C. Dollard, J.L. Wilson, L.M. Demeter, W. Bonnez, R.C. Reichman, T.R. Broker and L.T. Chow, Production of human

papillomavirus and modulation of the infectious program in epithelial raft cultures, *Genes & Development* **6** (1992), 1131–1142.

[8] N. Dyson, P.M. Howley, K. Munger and E. Harlow, The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product, *Science* **243** (1989), 934–937.

[9] W. El-Deiry, T. Tokino, V. Velculescu, D. Levy, R. Parson, J. Trent, D. Lin, W. Mercer, K. Kinzler and B. Vogelstein, WAF1, a potential mediator of p53 tumor suppression, *Cell* **75** (1993), 817–825.

[10] M.G. Frattini, H.B. Lim, J. Doorbar and L.A. Laimins, Induction of human papillomavirus type 18 late gene expression and genomic amplification in organotypic cultures from transfected DNA templates, *Journal of Virology* **71** (1997), 7068–7072.

[11] P.M. Howley, Papillomavirinae: the viruses and their replication, in: *Fundamental Virology*, B.N. Fields, D.M. Knipe and P.M. Howley, eds, Raven Press, New York, 1996, pp. 947–978.

[12] J.M. Huibregtse, M. Scheffner and P.M. Howley, A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18, *EMBO Journal* **10** (1991), 4129–4135.

[13] V.R. Iyer, M.B. Eisen, D.T. Ross, G. Schuler, T. Moore, J.C.F. Lee, J.M. Trent, L.M. Staudt, J.J. Hudson, M.S. Boguski, D. Lashkari, D. Shalon, D. Botstein and P.O. Brown, The transcriptional program in the response of human fibroblast to serum, *Science* **283** (1999), 83–87.

[14] T. Kartasova and P. Van De Putte, Isolation, characterization, and UV-stimulated expression of two families of genes encoding polypeptides of related structure in human epidermal keratinocytes, *Molecular and Cellular Biology* **8** (1988), 2195–2203.

[15] L.A. Laimins, The biology of human papillomaviruses: from warts to cancer, *Infectious Agents and Diseases* **2** (1993), 74–86.

[16] P. Leechanachai, L. Banks, F. Moreau and G. Matlashewski, The E5 gene from human papillomavirus type 16 is an oncogene which enhances growth factor-mediated signal transduction to the nucleus, *Oncogene* **7** (1992), 19–25.

[17] H. Leffers, P. Madsen, H.H. Rasmussen, B. Honore, A.H. Andersen, E. Walbum, J. Vandekerckhove and J.E. Celis, Molecular cloning and expression of the transformation sensitive epithelial marker stratifin, *Journal of Molecular Biology* **231** (1993), 982–998.

[18] S. Li, S. Labrecque, M.C. Gauzzi, A.R. Cuddihy, A.H. Wong, S. Pellegrini, G.J. Matlashewski and A.E. Koromilas, The human papilloma virus (HPV)-18 E6 oncoprotein physically associates with Tyk2 and impairs Jak-STAT activation by interferon-alpha, *Oncogene* **18** (1999), 5727–5737.

[19] D.R. Lowy, R. Kirnbauer and J.T. Schiller, Genital human papillomavirus infection. [Review], *Proc. Natl. Acad. Sci. USA* **91** (1994), 2436–2440.

[20] L.G. Martin, G.W. Demers and D.A. Galloway, Disruption of the G1/S transition in human papillomavirus type 16 E7-expressing human cells is associated with altered regulation of cyclin E, *Journal of Virology* **72** (1998), 975–985.

[21] A.A. McBride, H. Romanczuk and P.M. Howley, The papillomavirus E2 regulatory proteins, *Journal of Biological Chemistry* **266** (1991), 18411–18414.

[22] P.B.J. McCray and L. Bentley, Human airway epithelia express a β-defensin, *America Journal of Respiratory Cell Molecular Biology* **16** (1997), 343–349.

[23] C. Meyers, M.G. Frattini, J.B. Hudson and L.A. Laimins, Biosynthesis of human papillomavirus from a continuous cell line upon epithelial differentiation, *Science* **257** (1992), 971–973.

[24] A.E. Parker, G.N. Wheeler, J. Arnemann, S.C. Pidsley, P. Ataliotis, C.L. Thomas, D.A. Rees, A.I. Magee and R.S. Buxton, Desmosomal glycoproteins II and III, *The Journal of Biological Chemistry* **266** (1991), 10438–10445.

[25] L.V. Ronco, A.Y. Karpova, M. Vidal and P.M. Howley, Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity, *Genes and Development* **12** (1998), 2061–2072.

[26] M. Scheffner, J.M. Huibregtse, R.D. Vierstra and P.M. Howley, The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53, *Cell* **75** (1993), 495–505.

[27] M. Scheffner, B.A. Werness, J.M. Huibregtse, A.J. Levine and P.M. Howley, The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53, *Cell* **63** (1990), 1129–1136.

[28] R. Thweatt, C.K.J. Lumpkin and S. Goldstein, A novel gene encoding a smooth muscle protein is overexpressed in senescent human fibroblasts, *Biochemical and Biophysical Research Communications* **187** (1992), 1–7.

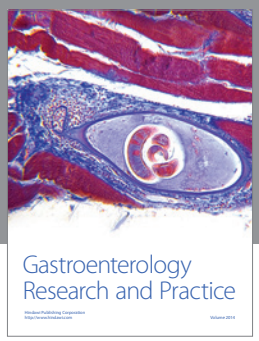
[29] M.L.A. Virata, R.M. Wagner, D.A.D. Parry and K.J. Green, Molecular structure of the human desmoplakin I and II amino terminus, *Proc. Natl. Acad. Sci. USA* **89** (1992), 544–548.

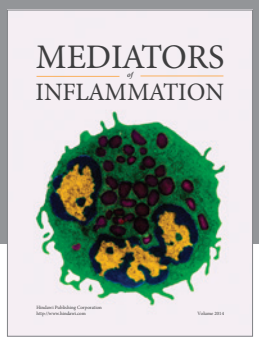
[30] J. Wang, L.Y. Xie, S. Allan, D. Beach and G.J. Hannon, Myc activates telomerase, *Genes and Development* **12** (1998), 1769–1774.

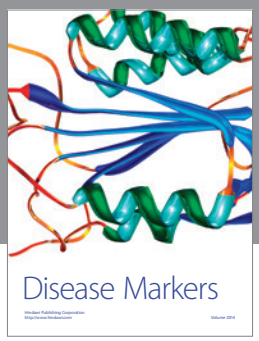
[31] D. Yang, O. Chertov, S.N. Bykovskaia, Q. Chen, M.J. Buffo, J. shogan, M. Anderson, J.M. Schroder, J.M. Wang, O.M.Z. Howard and J.J. Oppenheim, β-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6, *Science* **286** (1999), 525–528.

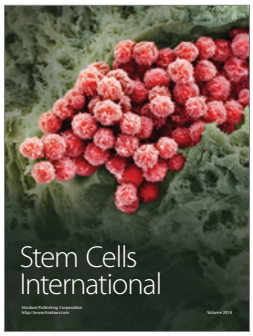
[32] H. Zhu, J.P. Cong, G. Mamtora, T. Gingeras and T. Shenk, Cellular gene expression altered by human cytomegalovirus: global monitoring with oligonucleotide arrays, *Proc. Natl. Acad. Sci. USA* **95** (1998), 14470–14475.

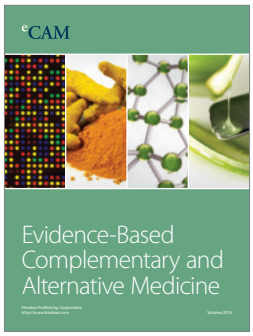
[33] H. zur Hausen and E.M. de Villiers, Human papillomaviruses. [Review], *Annu. Rev. Microbiol.* **48** (1994), 427–447.

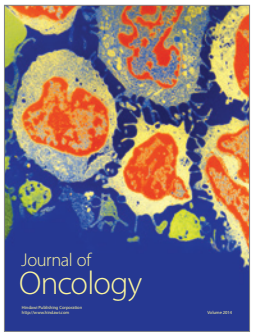


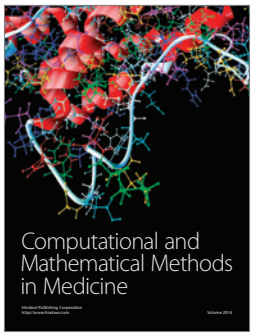


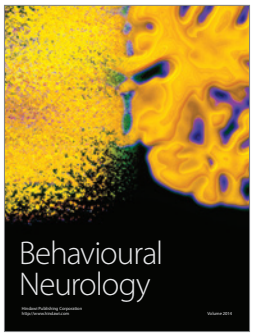


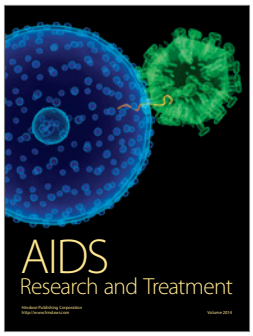


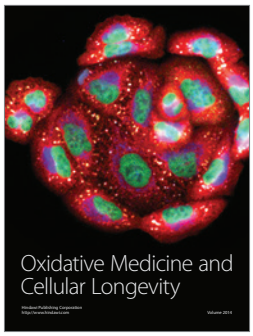












Hindawi

Submit your manuscripts at
http://www.hindawi.com